Cystic echinococcosis and other helminth infections of wild boar in northeastern and northwestern regions of Tunisia

Lahmar, Samia ; Torgerson, Paul R ; Mhemmed, Hana ; Tizaoui, Lamia ; Mhadhbi, Néjib ; Bani, Abdelkader ; Driss, Hanan ; Ghrissi, Nébiha ; Makhzoumi, Manel ; Ben Houidi, Amel ; Dhibi, Mokhtar ; Said, Yousra ; Pozio, Edoardo ; Boufana, Belgees

DOI: https://doi.org/10.1017/s0031182019000532

Cystic echinococcosis and other helminth infections of wild boar in northeastern and northwestern regions of Tunisia

Samia Lahmar[1*], Paul R. Torgerson[2], Hana Mhemmed[1], Lamia Tizaoui[1], Néjib Mhadhbi[1], Abdelkader Bani[1], Hanan Driss[1], Nébiha Ghrissi[1], Manel Makhzoumi[1], Amel Ben Houidi[1], Mokhtar Dhibi[1], Yousra Said[1], Edoardo Pozio[3] and Belgees Boufana[4]

[1] Parasitology Laboratory, National School of Veterinary Medicine, 2020 Sidi Thabet, Tunisia.

[2] Section of Epidemiology, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland

[3] Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy.

[4] National Wildlife Management Centre, National Reference Laboratory for *Trichinella* and *Echinococcus*, Animal and Plant Health Agency, York, U.K.

*** Author for correspondence**: Samia Lahmar, E-mail: drlsamia@yahoo.fr

Abstract

This study identified helminth species of wild boar (*Sus scrofa*) originating from northeastern and northwestern regions of Tunisia using 297 lungs, 297 livers, 264 intestinal tracts, 120 samples of muscle tissue (tongue, masseter, diaphragm, inter-costal) and 232 faecal samples derived from a total of 591 animals. Host gender was registered for the lung and liver wild boar group, which included 163 males and 134 females. All animals, excluding those used to retrieve muscular samples, were classified into 3 age classes, < 2 (n = 212), 2-3 (n = 208) and ≥ 4 years old (n = 141).

Helminth fauna of the examined wild boar included 14 parasite species: one trematode (adult, *Brachylaemus suis*), three cestodes (metacestodes of *Echinococcus granulosus*, *Taenia hydatigena* cysticercus, adult, *Hymenolepis diminuta*), nine nematodes (adults of *Metastrongylus apri, Metastrongylus pudendotectus, Ascarops strongylina, Globocephalus*

This is an Accepted Manuscript for Parasitology. This version may be subject to change during the production process. DOI: 10.1017/S0031182019000532

urosubulatus, Physocephalus sexalatus, Gnathostoma hispidum, Gongylonema pulchrum and eggs of *Strongyloides ransoni* and *Capillaria* spp.) and one acanthocephalan (adult, *Macracanthorhynchus hirudinaceus*). *Trichinella* larvae were not recovered from any of the 30 wild boar examined. Results showed a 73.5% global prevalence of infection with visceral helminths, 67.3% of which were lung and hepatic infections and 80.3% of helminths were recovered from the gastrointestinal tract. The most prevalent parasite was *M. hirudinaceus* (61.7%) while the highest intensity of infection was observed for *Metastrongylus* spp. The most prevalent cestode was *E. granulosus* (18.9%). This is the first detailed study on helminth infections of wild boar from a North African country.

Key Words: *Sus scrofa*, helminths, necropsy, epidemiology, Tunisia

Introduction

Wild boar populations occur throughout the steppe and forest regions of the world (Oliver and Leus, 2008). They remain widely distributed and often locally abundant but are also increasing in numbers in several countries, spreading into northern regions. As a result of their depredation on crops, wild boar are not appreciated in many countries, where they are managed as a game animal. In addition, they harbour many infectious agents (bacteria, viruses, and parasites) transmissible to other animal species and humans, through contaminated food and/or the environment (Fredriksson-Ahomaa, 2018). Changes of human habitation to suburban areas, increased use of land for agricultural purposes and deforestation have all increased chances of wild boar contact with domestic animals and humans (Meng *et al*., 2009). Wild boar, *Sus scrofa algira* and the Barbary wild boar, *Sus scrofa barbarus* are two species native to North African countries including Morocco, Algeria and Tunisia (http://fr.wikipedia.org). Wild boar is among the most abundant and economically important wildlife species in Tunisia. Despite their ubiquitous presence across most of Tunisia, accurate figures of wild boar population are not currently available. However, their observed numbers during the hunting season (September-April) for the period between1991-2009 was estimated to be approximately 10,293 animals and the mean annual number hunted during the same period was 2,437 wild boar (Karem *et al.*, 2009). Studies on parasitic infections of wildlife species are important to evaluate the extent to which wild animals may potentially serve as source of disease transmission to livestock and humans. Information on the helminth parasitic fauna of wild boar in Tunisia remains limited. Being omnivorous, wild boar feed on small mammals, invertebrates, carrions and plants and could therefore potentially play an important role in the transmission of zoonotic parasites (Solaymani-Mohammadi *et al.*, 2003). Wild boar from Tunisia were molecularly confirmed to harbour *Echinococcus granulosus* sensu stricto (s.s), although their epidemiological role in the spread of cystic echinococcosis (CE) is

unknown (Boufana *et al.*, 2014). Due to their encroachment on human habitation, where they have been observed in Tunisia to roam freely around houses and suburban areas, wild boar may serve as reservoirs for human helminthiases.

The aims of this survey were to describe the helminth fauna of wild boar from northeastern and northwestern Tunisia; to determine parasite prevalence and intensity, and analyze their association with gender and age with special reference to CE infection pressure.

Materials and methods

A total of 591 North African wild boar (*S. scrofa*) shot during the 2008-2013 hunting seasons in northeast (Ariana, Mabtouh, Bou Argoub, Djebel Ressas) and northwest (Bou Salem, Bou Arada, Gaâfour, Fernana, Touaref) Tunisia were included in this study. The type of organs recovered from each wild boar carcass were determined by the hunters and thus the investigated sample was conveniently divided into 3 groups. Wild Boar Group 1 (WBG1) included 297 wild boar carcasses collected between 2008 and 2011, which were used to examine the lungs, liver and abdominal cavity for the presence of helminth species. Wild Boar Group 2 (WBG2) consisted of 264 animals obtained during 2009-2013 hunting seasons which were used to identify gastrointestinal helminths. Wild Boar Group 3 (WBG3) consisted of 30 wild boar shot between 2010-2012 which were specifically used for the detection of *Trichinella* species. Age was estimated for animals within WBG1 and WBG2 using the dental formula described by Oroian *et al.* (2010). Animals were classified into 3 age classes, < 2 (n = 212), 2-3 (n = 208) and ≥ 4 years old (n = 141). Among the WBG1 and WBG2 animals, 305 and 256 originated from the northeast and the northwest regions of Tunisia, respectively. Host gender was registered only for WBG1 which included 163 males and 134 females. Wild boar carcasses and/or organs were transported to the Parasitology Laboratory of the Veterinary School, Sidi Thabet, Tunisia were analysis was carried out.

Lung and hepatic helminths

Individual samples of lungs and livers from 297 necropsied wild boar (WBG1) were examined for lungworms, *E. granulosus* metacestodes and *Taenia hydatigena* larvae. The trachea, large bronchi and bronchioles were opened using a pair of scissors and inspected for the presence of metastrongylids. Lungs were then cut into small pieces and soaked in 0.9% NaCl. After discarding large pieces of tissue, lungworms were collected in the sediment and mounted in chloral lactophenol solution (44%). The shape and length of spicules as well as the size and ray arrangement of the copulatory bursa were used to identify metastrongylid species as previously described (Skrjabin *et al*., 1952; Gassó *et al*., 2014).

E. granulosus hydatid cysts were identified by gross examination and palpation of the lungs and livers. Livers and lungs were then cut into thin slices and the number, size, localization and type of cysts were recorded. Hydatid fluid was aspirated from each pulmonary and hepatic viable cyst and examined to determine protoscoleces fertility rate. The mean fertility rate corresponded to the total number of the extrapolated protoscoleces/ml in each cyst divided by the total number of fertile cysts. Viability of protoscoleces was assessed for each fertile cyst per animal and organ using the method described by Smyth and Barrett (1980). A drop of 0.2% aqueous eosin solution was added to an equal volume of protoscoleces retrieved from a drop of cyst fluid sediment. A total of 100 protoscoleces in the sample was then scored as stained (indicating that the protoscoleces were dead) or unstained (indicating that the protoscoleces were viable). The mean percentage of protoscoleces viability was calculated by dividing the total number of viable protoscoleces of each cyst by the total examined protoscoleces in all fertile cysts multiplied by 100. Caseous hydatid cysts were considered infertile.

Gastro-intestinal helminths

Abdominal viscera were removed from 264 necropsied wild boar (WBG2). The oesophagus, stomach and intestinal tract of each animal were separated then opened longitudinally and carefully inspected for the presence of worms; the contents were washed in 0.9% NaCl and repeatedly rinsed through 100 mesh standard sieves which allowed the collection of helminths. Nematodes and trematodes were cleared using chloral lactophenol solution (44%) and identified under a light microscope based on figures and descriptions provided by Anderson (1992); they were then counted and stored in 70% alcohol. Identification of cestodes was based on the size and morphology of the proglottids following staining with 1% acetocarmine solution (Khalil *et al*., 1994). Worm size, shape and the arrangement of the proboscis hooks were used in the identification of acanthocephalans.

Collection and examination of faecal samples

Of the 264 wild boar within WBG2, 232 faecal samples were collected directly from the rectum of each animal and examined for the presence of parasite eggs and larvae using the qualitative flotation method. From each sample, 10 grams of faeces were weighed and mixed with 75 ml of saturated $MgSO_4$ solution. Eggs were isolated onto glass cover slips superimposed on convex meniscus for 15 minutes. Recovered eggs were identified based on their morphology (shape, envelope structure, number and size of blastomeres or presence of larvae) and biometrics (Thienpont *et al*., 1986; Georgi and McCulloch, 1989).

Examination of wild boar muscle for larvae of Trichinella

A total of 120 muscle samples from 30 wild boar (WBG3) (four muscle samples per wild boar) were used to investigate the presence of *Trichinella* larvae. This was carried out using trichinoscopy and by artificial digestion (artificial gastric juice) using 5 grams of the tongue,

masseter, pillar of diaphragm and inter-costal muscles. About 80 grams of muscle tissue was collected from each animal including 20 grams from the tongue, 20 grams from the masseter, 20 grams from the diaphragm and 20 grams from the inter-costal muscle. The trichinoscopy was performed by compressing 28 small pieces of muscle tissue, about 2 mm by 10 mm, with a total weight of approximately 0.5 grams from each sample. Muscle pieces were compressed between two glass plates until they become translucent, and examined using a stereomicroscope (Nöckler *et al*., 2000). Artificial digestion for the detection of muscle larvae was performed using the magnetic stirrer method (Gamble *et al*., 2000; Mayer-Scholl *et al*., 2017).

Data analysis

Data was analyzed in R (R Core Team, 2017). The probability distribution of the abundance of each parasite in its host was analyzed through maximum likelihood. Count models analyzed included Poisson, negative binomial and zero inflated models. The most parsimonious model was used for further analysis. The abundance of each parasite was analyzed according to age and geographic origin of host (northeast or northwest Tunisia). Generalized linear models (GLM) (with or without zero inflation) were utilized to explore these relationships. Generalized additive models (GAM) were also used to model the variations of abundance and prevalence of *E. granulosus* with age in wild boar. Finally, variations in abundance and prevalence of echinococcosis was examined according to existing models and explored for evidence of parasite-induced immunity or variations in infection pressure. Thus, the variation in abundance *m (t)* with age of the host *t* could be modelled as follows

$$m(t) = \frac{ght}{g + ah} + \frac{ah^2}{(g + ah)^2}[1 - exp\{-(g + ah)t\}]$$

where h is the infection pressure in cysts per year, a is the probability of inducing immunity, and g is the rate of loss of immunity (Roberts *et al*., 1986; Torgerson and Heath, 2003). Once infected, an animal is assumed to be infected for life. In the absence of parasite-induced host immunity (i.e. $a = 0$), this can be expressed simply as:

$$m(t) = ht$$

Likewise, the prevalence p (t) can be expressed as:

$$p(t) = k(1 - exp\{-bt\})$$

where b is the infection pressure in exposures per year and k is a constant that reduces the asymptotic prevalence below 1 in the presence of parasite-induced host immunity. The data was fitted to the models using maximum likelihood techniques to find the most likely parameter values and assess the goodness of fit to model.

Statistical analysis of prevalence of infection used Fisher's exact and χ^2 tests (Schwartz, 1993), with a p-value of < 0.05 considered indicative of a statistically significant difference.

Results

Global infection

A total of 32,060 recovered helminths belonging to fourteen species (adults of *Brachylaemus suis, Hymenolepis diminuta, Metastrongylus apri, Metastrongylus pudendotectus, Ascarops strongylina, Globocephalus urosubulatus, Physocephalus sexalatus, Gnathostoma hispidum, Gongylonema pulchrum* and *Macracanthorhynchus hirudinaceus;* larval stages of *E. granulosus* and *T. hydatigena*; eggs of *Strongyloides ransoni* and *Capillaria* spp.) were recovered in this study. The visceral necropsy of the examined wild boar (n = 561) revealed a global infection prevalence of 73.5% (± 0.019) (412/561) with a global mean intensity and mean abundance of 77.81 helminths per infected animal and 57.14 parasites per examined wild boar, respectively (Table 1). The most prevalent helminth was *M. hirudinaceus* while lung nematodes (*Metastrongylus sp*.) were the most abundant. The most frequently infected

tissues were the lungs (60.3%), followed by the stomach (21.5%), small intestine (17.7%), liver (0.4%) and oesophagus (0.02%) (Table 2). Coproscopic examination of faecal samples collected from WBG2 revealed the presence of two other nematode species not detected by necropsy namely, *Capillaria* spp. and *Strongyloides ransoni* in one (1/232) and two wild boar (2/232), respectively. *Trichinella* larvae were not detected in the muscular tissues of wild boar within WBG3. Wild boar from the northwest (73.8%) were similarly infected with visceral helminths as those from the northeast (73.1%) regions (χ^2= 0.036; p= 0.84) (Table 3). The global prevalence of infection with visceral helminths relative to host age was significantly different in the examined three age groups, increasing with age from 66.5% (± 0.032) to 85.1% (± 0.03) for wild boar < 2 and ≥ 4 years old, respectively (χ^2= 15.13; p= 0.0005) (Table 4).

Most parasites had an over dispersed distribution which could be best modelled by either a negative binomial or zero inflated negative binomial distribution. For *T. hydatigena* and *Gnathostoma* species the small number of positive counts were single parasites, thus it was not possible to conclude that these parasites had an over dispersed distribution and a Poisson distribution was assumed (Table 5).

Prevalence and intensity of lung, liver and abdominal cavity helminths

Among the 297 animals in WBG1 investigated for lung and hepatic helminth infections, 200 wild boar were infected (67.3% ± 0.027) with a mean intensity of 97.45 and a mean abundance of 65.62 worms per host (Table 1). Animals from the northeast (73.1%) were more infected than those from the northwest (63.5%) (χ^2= 3; p= 0.08) (Table 3). Prevalence of infection with pulmonary and hepatic helminths increased significantly with age from 58.3% to 63.2% and 83.9% in animals aged < 2, 2-3 and ≥ 4 years old, respectively (χ^2= 15.92; p= 0.0003) (Table 4). No significant difference was observed between prevalence in female (80.6

±0.034) and male wild boar (73 ± 0.035) (χ^2= 2.35; *p*= 0.125) nor between the abundance of infection with lung and hepatic helminth species (*p* = 0.128) (Table 6).

Three parasite species were recovered from the lungs, liver and abdominal cavity namely, *Metastrongylus* spp., *E. granulosus* metacestodes and larvae of *T. hydatigena. Metastrongylus* spp. (*M. apri* and *M. pudendotectus*) were the most frequent nematode species (60.3% ± 0.028) isolated from the bronchi and bronchioles. Prevalence was similar for both species (60.3% ± 0.028). However, *M. apri* showed a higher intensity of infection (123.51 worms) than *M. pudendotectus* (93.73 worms per infected animal) (Table 2). A statistically significant difference in the prevalence was observed between the two studied regions for *Metastrongylus* spp. infection (χ^2= 6.18; *p*= 0.01) and the mean intensity was higher in the northwest (118.81) than in the northeast (93.84) of Tunisia (Table 3). Prevalence of these respiratory strongyles was higher in female 61.9% (± 0.042) than male wild boar 58.9% (± 0.039) (χ^2= 0.28; *p*= 0.59) as well as the abundance (73.4 for females; 57.28 for males) (*p*= 0.12) (Table 6). Prevalence of metastrongylids increased significantly with age from 46.1% (± 0.046) to 71.3% (± 0.049), in wild boar <2 and >4 years old, respectively (χ^2= 16.05; *p*= 0.0003) (Table 4). Also, the intensity of *Metastrongylus* increased with age. *Metastrongylus* had a zero-inflation which decreased with age and was higher in the northwest (Table 5).

Echinococcus granulosus was the most prevalent cestode recorded in 18.9% (± 0.023) of the 297 examined wild boar with a mean intensity of 4.58 worms per infected host and an abundance of 0.86 worms per examined host (Table 1). The liver and lungs were the only infected organs with co-infection of hepatic and pulmonary tissues encountered in 5.7% (17/297) of cases, and observed in all age-groups with 2.6%, 5.3% and 10.3% infection recorded for <2, 2-3 and ≥ 4 year olds respectively. There were no significant differences in prevalence rates between hepatic hydatid cysts (12.1%) and pulmonary cysts (12.5%) (χ2= 0.015; *p*= 0.9) nor in the abundance of *E. granulosus* larvae in the two organs (0.42 and 0.44

cyst per examined animal) (p= 0.93) (Table 2). There was a nonlinear relationship with age both in prevalence and abundance of *E. granulosus* hydatid cysts (Fig. 1 & 2). The variation in age prevalence did not give a good fit to the standard mathematical model, with a reduction in prevalence between ages 3 and 5 and a decrease in older animals (Fig. 1). Likewise, the age abundance variation gave a poor fit (Fig. 2). The fit given by the GAM model suggested that hydatid cyst abundance peaked at approximately 3 years and decreased as the animals became older.

No significant difference was noted in CE rates between males (16.6% ± 0.029) and females (21.6% ± 0.036) ($\chi^2_=$ 1.23; p= 0.26) neither in abundance (0.9 for males; 0.81 for females) (p= 0.83) (Table 6). Differences in CE prevalence between regions of the northeast (19.3%) and the northwest (18.5%) were not statistically significant ($\chi^2_=$ 0.028; p= 0.86) (Table 3). The mean number of cysts per infected wild boar as well as abundance were highest (6.05; 1.27) in animals aged between 2-3 years old and lower in the two other age groups (<2 and ≥4 years) varying between 4.25 – 0.97 and 3.54 – 0.44 cysts per infected animal, respectively (Table 4). Cyst size varied between 5 and 60 mm in diameter for each animal. The diameter of hepatic and pulmonary cysts ranged from 11 to 20 mm for 53 cysts (20.6%), 21 to 60 mm for 39 cysts (15.2%) while 165 cysts (64.2%) were less 10 mm wide.

Of the 257 recovered hydatid cysts, 238 (92.6%) were viable including 6 (2.3%) fertile cysts while 19 (7.4%) cysts were caseous. Animals aged between 2-3 years had the highest proportion of viable (43.2%) and dead (3.9%) cysts. The percentage of viable cysts followed a positive linear correlation with age (R^2 = 0.2892; y = 0.0681x + 0.1724). Lung hydatid cysts (3.8%) were more fertile than their hepatic counterparts (0.8%). Mean fertility rates of the 6 fertile hydatid cysts were estimated to be 850, 950, 1450, 1600 and 1750 for the five pulmonary fertile cysts and 200 protoscoleces per ml of hydatid cyst fluid for the unique fertile hepatic cyst. The fertility rate was highest in cysts measuring between 21 and 60 mm in

diameter. No fertile cyst was detected in wild boar <2 years old whereas the mean fertility rate increased with age from 1950 to 4850 protoscoleces per ml in 2-3 and ≥ 4 years age groups, respectively. Of the total viable hydatid cysts, germinal layers and protoscoleces of 27 hydatid cysts (12 hepatic and 15 pulmonary) removed from 23 wild boar originating equally from the northwest and the northeast studied areas, were characterized at the molecular level and reported to be those of *E. granulosus* s.s (Boufana *et al*., 2014).

Taenia hydatigena larvae were encountered in the liver and the abdominal cavity of 4.4% (± 0.011) of the examined wild boar with a mean intensity and a mean abundance of 1 and 0.04 larvae, respectively (Tables 1 & 5). Infection in wild boar originating from the northeast was higher (6.7%) than that observed in animals from the northwest (2.8%) (Table 3) (χ^2 =2.6; p= 0.1). With respect to age, older wild boar (≥ 4 years) were less infected than other age groups but no significant difference was noted between the prevalence of the 3 age classes (F.E.T.; p = 0.46) (Table 4). The prevalence of *T. hydatigena* larvae was almost similar in female (4.5% ± 0.018) and male wild boar (4.3% ± 0.016) (χ^2 = 0.005; p= 0.93) as well as the mean abundance in male (0.042) and female (0.044) (p= 0.93) animals (Table 4).

Prevalence and intensity of gastrointestinal helminths

Of the 264 wild boar examined for gastrointestinal parasites (WBG2), 212 animals were found to be infected with at least one helminth species, giving an overall prevalence of 80.3% (± 0.024), a mean intensity of 59.29 worms per infected animal and an abundance of 47.61 (Table 1). The global prevalence of infection with gastrointestinal helminths was significantly higher in animals from the northwest (97.4%) than those from the northeast (73.1%) (χ^2= 20.54; p= 0.0000) as well as the mean intensity of infection with 95.42 and 39.10 worms per infected animal, respectively (Table 3). In addition, the global prevalence of gastrointestinal helminths increased with host age from 76.3% (± 0.043) in the youngest to 87% (± 0.046) in older animals (χ^2= 2.54; p= 0.28) (Table 4). Eight parasite species were detected including one

trematode (*B. suis*), one cestode (*H. diminuta*), five nematodes (*A. strongylina*, *G. urosubulatus*, *G. pulchrum, P. sexalatus, G. hispidium*) and one acanthocephalan (*M. hirudinaceus*). The most frequently found gastrointestinal parasites were *M. hirudinaceus* (61.7% ± 0.030), *A. strongylina* (54.9% ± 0.031), *G. urosubulatus* (26.5% ± 0.027) and *B. suis* (21.2% ± 0.025) (Table 1). The overall mean intensities of gastrointestinal helminth species were high for *B. suis* (52.73), *A. strongylina* (46.92), *G. urosubulatus* (34.42) and low for *P. sexalatus* (8.22), *H. diminuta* (2), *M. hirudinaceus* (1.47) and *G. hispidium* (1). The highest gastrointestinal helminth abundance was observed for *A. strongylina* (25.77 worms) with the total number ranging from 1 to 495 worms. *Ascarops strongylina* had an increased abundance and prevalence in the northwest compared to northeast regions (Tables 3 & 5). The highest gastrointestinal helminth intensity was observed for *B. suis* (52.73) with the total number ranging from 1 to 564 worms whereas the lowest mean intensity and mean abundance were recorded for *G. hispidium* (1 and 0.003) (Tables 1 & 2). In the northeast, wild boar was infected with all the gastrointestinal helminth species identified here while *G. hispidium* and *H. diminuta* were absent in animals originating from the northwest regions (Table 3). Gastrointestinal helminths were encountered in all age groups except for *G. pulchrum* which was absent in wild boar < 2 years old; *H. diminuta* which was recovered only from the youngest age group (<2 years) and *G. hispidium* identified in one animal aged between 2-3 years old. A significant difference was observed in prevalence between the three age groups, except for *G. urosubulatus* ($\chi^2_=$ 10.26; p= 0.005) and *B. suis* ($\chi^2_=$ 5.99; p= 0.04) (Table 4). *Globocephalus urosubulatus* had a zero-inflated distribution with the zero-inflation decreasing with age (and hence prevalence increasing with age) (Table 5).

Discussion

The present study investigated for the first time the visceral and muscular helminth infection status of Tunisian wild boar. Wild boar is a known reservoir host for helminth species of public health significance (Sánchez *et al*., 2012; Umhang *et al*., 2014). In the present study, seven zoonotic helminths were hosted by 43.3% (243/561) of the examined wild boar including *E. granulosus*, *H. diminuta*, *M. hirudinaceus, G. pulchrum, S. ransoni, M. apri* (or *M. elongatus*) and *G. hispidium.* Of the 14 helminth species identified in this study, *E. granulosus, T. hydatigena* and *G. pulchrum* were previously recovered from domestic ruminants (Souilem, 1986; Maâmouri, 2005; Lamouchi, 2009; Selmi, 2009) and wild herbivore hosts (Boufana *et al*., 2015) in Tunisia. The remaining species of helminths with the exception of *H. diminuta,* are strictly wild boar-specific.

Our results indicated a diversified visceral helminth fauna infecting wild boar with lung nematodes, *M. apri* and *M. pudendotectus* showing the highest prevalence (60.3% ± 0.028). High prevalence of these respiratory strongyles was reported in wild boar from France (92%), Estonia (82%), southwestern Iran (68%) and Brazil (60%) (Humbert and Henry 1989; Jarvis *et al*., 2007; da silva and Müller, 2013; Mansouri *et al*., 2016) whereas a lower prevalence of 48.6% and 41.1% was recorded from Poland and Spain respectively (Popiolek *et al*., 2010; Garcia-Gonzălez *et al*., 2013). In this study, prevalence and intensity of infection with *Metastrongylus* increased with host age whereas in France and Spain prevalence was greater in young (<1 year) wild boar (Humbert and Henry, 1989; Garcia-Gonzălez *et al*., 2013). Nematodes of genus *Metastrongylus* are found in wild boar worldwide. They may cause dyspnea, bronchopneumonia, tissue damage and secondary bacterial and viral infections. Respiratory disorders due to these parasites are a principal factor of permanent weight loss and mortality of wild and domestic pigs (Syrjälä *et al*., 2010). *Metastrongylus* spp. have an indirect life cycle; transmission occurs through ingestion of earthworms as intermediate hosts

in which infective larvae can survive for more than 1 month (Levine, 1980). Human infection with zoonotic *Metastrongylus* spp. (*Metastrongylus elongatus* and *Metastrongylus salmi*) although rare has been reported in the literature (Miloshev, 1956; Beaver *et al*., 1984; Calvopina *et al*., 2016).

Among the stomach nematodes found in the present survey, *A. strongylina* and *P. sexalatus* were reported in wild boar from France (Humbert and Henry, 1989) and Poland (Popiolek *et al*., 2010). *Physocephalus sexalatus* was also found in guts of free-roaming pigs from western Thailand (Chaisiri *et al*., 2017). The life cycle of *A. strongylina* and *P. sexalatus* involves sharing a common intermediate host with transmission to wild boar being achieved through the ingestion of dung-eating insects harboring the infective stage. *Strongyloides ransoni* is almost always an infection of young swine. The lower coproscopic prevalence of *S. ransoni* observed in this survey, may be a reflection of host age as all the wild boar examined were >1-year-old. In this study, all visceral helminths retrieved at necropsy were not detected by coprological analysis. This could be due to a low reproductive rate of helminth species, seasonal variations and/or host factors or due to our examination of a single faecal sample per animal. To assess helminth infections in wild boar, Gassó *et al.* (2015) recommended coprological examination over several days post-collection. However, necropsy remains as the gold standard technique for screening helminth fauna in hunted animals while coproscopy represents a supplementary test utilized to complement observed results. *Macracanthorhynchus hirudinaceus* encountered in the present investigation at high prevalence (61.7% ± 0.03) has been previously reported from necropsied wild canids and stray dogs in Tunisia (Lahmar *et al*., 2009a; 2017) as well as from swine in other countries (Eslami *et al*., 1992; Solaymani-Mohammadi *et al*., 2003; Foata *et al*., 2005; Senlik *et al*., 2011; Mansouri *et al*., 2016).

This is the first study that investigated *Trichinella* infection from wild boar in Tunisia. Although none of the wild boar examined in this study were infected, the lack of a positive result is not surprising, since the expected species, *Trichinella britovi* shows a very low infectivity to domestic and wild swine. In Europe for example, *Trichinella* infection prevalence in hunted wild boar in 2016 was 0.08% (EFSA, 2017). It follows that the examined sample size (n = 30) could have been too small to allow the detection of any infected animals. Encapsulated larvae of *Trichinella sp*. (thought to be those of *T. britovi*) were detected in Tunisian wild carnivores, including genet (*Genetta genetta*), jackal (*Canis aureus*), and mongoose (*Herpestes ichneumon*) (Fassbender and Mayer, 1974). However, no zoonotic human infections derived from wild populations have to date, been reported from Tunisia mainly because most of the population is Muslim who are prohibited from eating pork and do not normally consume meat of wild carnivores. Interestingly, the only known case of human trichinellosis in a 23-year-old Tunisian was related to the consumption of semi-raw equine meat (Marrakchi *et al*., 1989). In contrast, 6 outbreaks associated with a domestic pig and wild boar meat were reported from neighboring Algeria (Pozio, 2007). In addition, a single case was documented in an Algerian who consumed meat from a jackal (*C. aureus*) infected with *T. britovi* (Nezri *et al*., 2006). Further investigations using a larger sample size of wild boar from Tunisia are required.

In Tunisia, CE caused by metacestodes of *E. granulosus* sensu lato (G1-G3; G6) (Lahmar *et al.*, 2009b; Oudni-M'rad *et al*., 2016) is a major rural and urban zoonosis as a consequence of a predominantly domestic life-cycle between free roaming dogs and domestic intermediate hosts, and a secondary sylvatic life-cycle between wild canids and wild herbivores (Lahmar *et al*., 2009a; Boufana *et al*., 2015). In Tunisia, there are at least 12.6 human CE cases per 100, 000 inhabitants (Chahed *et al.*, 2015). Our results confirmed the important role of wild boar in the transmission of cystic echinococcosis (18.9%) in the northeastern and northwestern areas

of Tunisia acting as reservoirs of *E. granulosus* human infection through the dominant *E. granulosus* s.s. To the best of our knowledge, to date, only one study on *E. granulosus* infection in feral swine from North Africa (Algeria) has been published (Bentounsi *et al*., 2009). In other Mediterranean countries, a recent survey on wild boar from central Italy reported a lower prevalence (1.0%; 8/765) of infection with *E. granulosus* s.s. (G1) but the percentage of fertile hydatid cysts was higher than that reported here (Paoletti *et al*., 2018). Fertile cysts constitute a risk of contamination for domestic and wild definitive hosts through the consumption of wild boar carcasses.

The nonlinear relationship of *E. granulosus* with age both in prevalence and abundance observed in wild boar in this study was different to the previously reported linear increase of CE prevalence and abundance with age in all domestic intermediate hosts from Tunisia (Lahmar *et al*., 2013; 2014). The reasons for this apparent prevalence and abundance decrease of *E. granulosus* in older wild boar is not clear, but it is possible to hypothesize. This may suggest a parasite-induced host morbidity/mortality where wild boar could be regulated by natural predators. If the assumption is that once infected with a hydatid cyst, the animal remains infected for life, then there should not be a drop off in the prevalence (or abundance) in older animals, regardless of the presence or absence of immunity. So, it may be that there were variations in infection pressure across the different age cohorts or perhaps interestingly, there could be parasite-induced morbidity or mortality. The latter would make older (and heavily infected wild boar) less able to survive or susceptible to predation by for example golden jackals. Thus, old infected wild boars have died, whilst uninfected ones have survived for longer, thus resulting in the decrease in prevalence in the oldest cohort. The possibility of infected intermediate hosts having an increased susceptibility to predation has been suggested in at least one previous report. In a study of echinococcosis in moose in Canada, the pattern of cyst aggregation suggested that *Echinococcus*-infected moose may be more susceptible to

predation by wolves (Joly and Messier, 2004). The low prevalence of *T. hydatigena* larvae recorded in the present study, may reflect the roaming habits of wild boar in urban and agricultural areas contaminated with *T. hydatigena* eggs excreted by infected jackals and dogs. *Taenia hydatigena* larvae are known to cause huge economic losses in ruminants, pigs and wild boar due to their migration in their liver (Scala *et al*., 2015).

This study confirms the diversity of wild boar helminth fauna and the high level of infection with visceral parasites and the potential risks posed by these animals as reservoirs for zoonoses, especially CE. The recorded data justify further monitoring of the role of wild boar as a source of parasitic infection for domestic animals. The economic consequences related to parasite burdens that may lead to high morbidity and mortality and thus a decrease in wild boar population require further investigation.

Conflicts of interest statement. The authors declare no conflict of interests.

Financial support. This work was funded by The European research project PARAVAC (FP7-KBBE-2010-4-265862).

Ethical Standards. Not applicable.

References

Anderson, RC (1992) *Nematode Parasites of Vertebrates: Their Development and Transmission*. (1st Ed.), Wallingford, UK, CAB International University Press, 358- 359.

Beaver, PC, Jung, RC and Cupp, EW (1984) *Clinical Parasitology*. (9th Ed.). Philadelphia, PA : Lea and Febiger, 291-292.

Bentounsi, B, Meradi, S, Ayachi, A and Cabaret, J (2009) Cestodes of untreated large stray dog populations in Algeria: A reservoir for herbivore and human parasitic diseases. *Open Veterinary Sciences and Journal* **3**, 64-67.

Boufana, B, Lahmar, S, Rebaï, W, Ben Safta, Z, Jebabli, L, Ammar, A, Kachti, M, Aouadi, S, Craig, PS (2014) Genetic variability and haplotypes of *Echinococcus* isolates from Tunisia. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 1-9. https://doi.org/10.1093/trstmh/tru138

Boufana, B, Said, Y, Dhibi, M, Craig, PS and Lahmar, S (2015) *Echinococcus granulosus* sensu stricto (s.s.) from the critically endangered antelope *Addax nasomaculatus* in Tunisia. *Acta Tropica* **152**,112-115. https://doi.org/10.1016/j.actatropica.2015.08.015.

Calvopina, M, Caballero, H, Morita, T and Korenag, M (2016) Case report: Human pulmonary infection by the zoonotic *Metastrongylus salmi* nematode. The First reported case in the Americas Manuel. *The American Society of Tropical Medicine and Hygiene* **95**(4), 871–873. Doi :10.4269/ajtmh.16-0247

Chahed, MK, Bellali, H, Ben Alaya, N, Aoun, K and Zouari, B (2015) High risk areas for echinococcosis-hydatidosis in Tunisia. *La Tunisie Médicale* **93**, 33-7.

Chaisiri, K, Aueawiboonsri, S, Kusolsuk, T, Dekumyoy, P, Sanguankiat, S, Homsuwan, N, Peunpipoom G, Okamoto, M, Yanagida, T, Sako, Y and Ito, A (2017) Gastrointestinal helminths and *Taenia* spp. In parenteral tissues of free-roaming pigs (*Sus scrofa indicus*) from hill-tribe village at the western border of Thailand. *Tropical Biomedicine* **34**(2), 464-470.

da Silva, D and Müller, G (2013). Parasites of the respiratory tract of *Sus scrofa scrofa* (wild boar) from commercial breeder in southern Brazil and its relationship with *Ascaris suum*. *Parasitology Research* **112**(3), 1353-1356.

EFSA (2017) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. *EFSA Journal* **15** (12), 5077.

Eslami, A and Farsad-Hamdi, S (1992) Helminth parasites of wild boar, *Sus scrofa,* in Iran. *Journal of Wildlife Diseases* **28**(2), 316-318.

Fassbender, CP and Mayer, P (1974) Uber die verteilung von *Trichinella spiralis* in der Mushulatur einiger nordafrikanischer Carnivoren. *Deutsche Tierarztliche Wochenschrif* **81**, 284-287.

Foata, J, Culioli, JL and Marchand, B (2005) Helminth fauna of wild boar in Corsica. *Acta Parasitologica* **50**(2), 168-170.

Fredriksson-Ahomaa, M (2018) Wild Boar: A Reservoir of Foodborne Zoonoses. *Foodborne Pathogens and Disease*. DOI: 10.1089/fpd.2018.2512

Gamble, HR, Bessonov, AS, Cuperlovic, K, Gajadhar, AA, van Knapen, E, Noeckler, K, Schenone, H and Zhu, X (2000) International Commission on Trichinellosis: Recommendations on methods for the control of *Trichinella* in domestic and wild animals intended for human consumption. *Veterinary Parasitology* **93**, 393-408.

García-González, AM, Pérez-Martín, JE, Gamito-Santos, JA, Calero-Bernal, R, Alcaide Alonso, M and Frontera Carrión, EM (2013) Epidemiologic study of lung parasites (*Metastrongylus* spp.) in wild boar (*Sus scrofa*) in southwestern Spain. *Journal of Wildlife Diseases* **49**(1), 157-162. https://doi.org/10.7589/2011-07-217.

Gassó, D, Rossi, L, Mentaberre, G, Casas, E, Velarde, R, Nosal, P, Serrano, E and Segales, J, Fernandez-Llario, P, Feliu, C (2014) An identification key for the five most

common species of *Metastrongylus*. *Parasitology Research* **113**, 3495-3500. https://doi. 10.1007/s00436-014-4001-y.

Gassó, D, Feliu, C, Ferrer, D, Mentaberre, G, Casas-Díaz, E, Velarde, R, Fernández-Aguilar, X, Colom-Cadena, A, Navarro-Gonzalez, N, López-Olvera, J R, Lavín, S, Fenández-Llario, P, Segalés, J and Serrano, E (2015) Uses and limitations of faecal egg count for assessing worm burden in wild boars. *Veterinary Parasitology* **209** (1-2), 133-137. https://doi.org/10.1016/j.vetpar.2015.02.006

Georgi, JR and McCulloch, CE (1989) Diagnostic morphometry: Identification of helminth eggs by discriminant analysis of morphometric data. *Proceedings of The Helminthological Society of Washington* **56**(1), 44-57.

Humbert, JF and Henry, C (1989) Studies on the prevalence and the transmission of lung and stomach nematodes of the wild boar (*Sus scrofa*) in France. *Journal of Wildlife Diseases* **25**(3), 335-41. https://doi.org/10.7589/0090-3558-25.3.335

Järvis, T, Kapel, Ch, Moks, E, Talvik, H and Mägi , E (2007) Helminths of wild boar in the isolated population close to the northern border of its habitat area. *Veterinary Parasitology* **150**(4), 366-9.

Joly, DO and Messier, F (2004) The distribution of *Echinococcus granulosu*s in moose: evidence for parasite-induced vulnerability to predation by wolves ?. *Oecologia* **140**(4), 586-590.

Karem, A, Triplet, P and Lartiges, A (2009) *Faune sauvage de Tunisie : écotourisme, chasse et développement communautaire*. Ministère de l'Agriculture et des Ressources Hydrauliques, Direction Générale des Forêts, 128 p.

Khalil, LF, Jones, A and Bray, RA (1994) Keys to the Cestode Parasites of Vertebrates. Wallingford, UK, CAB International University Press., 751 pp.

Lahmar, S, Arfa, I, Ben Othmen, S, Jguirim, W, Said, Y, Dhibi, A and Boufana, B (2017) Intestinal helminths of stray dogs from Tunisia with special reference to zoonotic infections. *Parasitology Open* **3**, e18, 1-9. https://doi.org/ 10.1017/pao.2017.21

Lahmar, S, Boufana, B, Lahmar, S, Inoubli, S, Guadraoui, M, Dhibi, M, Bradshaw, H and Craig, PS (2009a) *Echinococcus* in the wild carnivores and stray dogs of northern Tunisia: the results of a pilot survey. *Annals of Tropical Medicine and Parasitology* **103**, 323-31.

Lahmar, S, Boufana, B, Jebabli, L, Craig, PS, Ayari, H, Basti, T, Dhibi, M and Torgerson, PR (2014) Modelling the transmission dynamics of cystic echinococcosis in donkeys of different ages from Tunisia. *Veterinary Parasitology* **205**, 119-124. http://dx.doi.org/10.1016/j.vetpar.2014.06.007

Lahmar, S, Rebaï, W, Boufana, B, Craig, PS, Ksantini, R, Daghfous, K, Chebbi, F, Ftériche, F, Bédioui, H, Jouini, M, Dhibi, M, Makni, A, Ayadi, MS, Ammous, A, Kacem, MJ and Ben Safta, Z (2009b) Cystic echinococcosis in Tunisia: analysis of hydatid cysts that have been surgically removed from patients. *Annals of Tropical Medicine & Parasitology* **103**, 7, 593–604.

Lahmar, S, Trifi, M, Ben Naceur S, Bouchhima, T, Lahouar, N, Lamouchi, I, Maâmouri, N, Selmi, R, Dhibi, M and Torgerson, PR (2013) Cystic echinococcosis in slaughtered domestic ruminants from Tunisia. *Journal of Helminthology* **87**, 318-325.

Lamouchi, I (2009) Contribution à l'étude épidémiologique de l'hydatidose et de la cysticercose hépato-péritonéale chez les dromadaires et les caprins abattus dans la région de Tozeur. *DVM Thesis, National School of Veterinary Medicine Sidi Thabet, Tunisia.*

Levine, ND (1980) *Nematode parasites of domestic animals and man*. (2nd Ed.), Burgess Publishing Company, Minneapolis, Minnesota, 477 pp.

Maâmouri, N (2005) Contribution à l'étude de l'hydatidose et de la cysticercose hépato-péritonéale chez les ruminants dans les abattoirs de Korba et Nabeul, Tunisie. *DVM Thesis, National School of Veterinary Medicine Sidi Thabet, Tunisia.*

Mansouri, M, Sakkari, B and Mowlavi, GR (2016) Helminth parasites of wild boars, *Sus scrofa*, in Bushehr Province, southwestern Iran. *Iran Journal of Parasitology* **11**(3), 377-82.

Marrakchi, R, Ben Romdhane, K and Touati, S (1989) Découverte fortuite du premier cas de trichinose en Tunisie. *Maghreb Médical*, n°**208**, Août1989.

Mayer-Scholl, A, Pozio, E, Gayda, J, Thaben, N, Bahn, P and Nöckler, K (2017) Magnetic stirrer method for the detection of *Trichinella* larvae in muscle samples. *Journal of Visualized Experiments* **3**, 121. https://doi.10.3791/55354.

Meng, XJ, Lindsay, DS and Sriranganathan, N (2009) Wild boars as sources for infectious diseases in livestock and humans. *Philosophical Transactions of the Royal Society B* **364**, 2697-2707. https://doi. 10.1098/rstb.2009.0086.

Miloshev, B (1956) Case of triple infection with *Metastrongylus elongatus, Thaeniarhynchus saginatus* and *Enterobius vermicularis* [in Bulgarian]. *Suvr Med.* (Sofiia) **7**, 94–97.

Nezri, M, Ruer, J, De Bruyne, A, Cohen-Valensi, R, Pozio, E and Dupouy-Camat, J (2006) Première observation d'un cas d'humain de trichinellose à *Trichinella britovi* en Algérie après consommation de viande de chacal (*Canis aureus*). *Bulletin de la Société de Pathologie Exotique* **99**, 94-95.

Nöckler, K, Pozio, E, Voigt, WP and Heidrich, J (2000) Detection of *Trichinella* infection in food animals. *Veterinary Parasitology* **93**, 335-350.

Roberts, MG, Lawson, JR and Gemmell, MA (1986) Population dynamics in echinococcosis and cysticercosis: mathematical model of the life-cycle of *Echinococcus granulosus*. *Parasitology* **92** (3), 621–641.

Oliver, W and Leus, K (2008) *Sus scrofa*. The IUCN Red List of Threatened Species 2008: e.T41775A10559847.http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T41775A10559 847.en

Oroian, TE, Oroian, RG, Paşca, I, Oroian, E and Covrig, I (2010) Methods of Age Estimation by Dentition in Sus scrofa ferus sp. *Bulletin UASVM Animal Science and Biotechnologies*, **67**(1-2), 1843-5262.

Oudni-M'rad, M, M'rad, S, Ksia, A, Lamiri, R, Mekki, M, Nouri, A, Mezhoud, H and Babba, H (2016) First molecular evidence of the simultaneous human infection with two species of *Echinococcus granulosus* sensu lato: *Echinococcus granulosus* sensu stricto and *Echinococcus canadensis*. *Parasitology Research* **115**(3), 1065-9. Doi.org/10.1007/s00436-015-4836-x.

Paoletti, B, Della Salda, L, Di Cesare, A, Iorio, R, Vergara, A, Fava, C, Olivastri, A, Dessì, G, Scala, A and Varcasia, A (2018) Epidemiological survey on cystic echinococcosis in wild boar from Central Italy. *Parasitology Research* 1-4. https://doi.org/10.1007/s00436-018-6112-3

Popiolek, M, Knecht, D, Szczesna-Stas Kiewicz, J and Czerwinsska-Rozalow, A (2010) Helminths of the wild boar *(Sus scrofa l.)* in natural and breeding conditions. *Bulletin of Veterinary Institute Pulawy* **54**, 161-166.

Pozio, E (2007) World distribution of *Trichinella* spp. infections in animals and humans. *Veterinary Parasitology* **149**, 3-21.

Sánchez, E, Cáceres, O, Náquira, C, Miranda, E, Samudio, F and Fernandes, O (2012) *Echinococcus granulosus* genotypes circulating in alpacas (*Lama pacos*) and pigs (*Sus scrofa*) from an endemic region in Peru. *The Memórias do Instituto Oswaldo Cruz* **107**(2), 275-8.

Scala, A, Pipia, AP, Dore, F, Sanna, G, Tamponi, C, Marrosu, R, Bandino, E, Carmona, C, Boufana, B and Varcasia, A (2015) Epidemiological updates and economic losses due to *Taenia hydatigena* in sheep from Sardinia, Italy. *Parasitology Research.* **114**(8), 3137-43.

Schwartz, D (1993) *Méthodes statistiques à l'usage des médecins et des biologistes*. (3rd Ed.), Paris : Flammarion.

Selmi, R (2009) Contribution à l'étude épidémiologique de l'hydatidose chez les caprins et dromadaires abattus dans la région de Gabès. *DVM Thesis, National School of Veterinary Medicine Sidi Thabet, Tunisia.*

Senlik, B, Cirak, VY, Girisgin, O and Akyol, CV (2011) Helminth infections of wild boars (*Sus scrofa*) in the Bursa province of Turkey. *Journal of Helminthology* **85**(4), 404-8. https://doi.org/10.1017/S0022149X1000074X.

Skrjabin, KI, Shikhobolova, NP, Schulz, RS, Popova, TI, Boev, SN and Delyamure, SL (1952) *Strongylata*. In: Skrjabin K.I. (Ed.) *Essentials of nematology*. Academy of Sciences, Moscow (in Russian), (English translation, 1961, by Israel program for scientific translations, Jerusalem).

Smyth, JD and Barrett, NJ (1980) Procedures for testing the viability of human hydatid cysts following surgical removal, especially after chemotherapy. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **74**, 649-652.

Solaymani-Mohammadi, S, Mobedi I, Rezaian, M, Massoud, J, Mohebali, M, Hooshyar, H, Ashrafi, K and Rokni, MB (2003) Helminth parasites of the wild boar, *Sus scrofa,* in Luristan province, western Iran and their public health significance. *Journal of Helminthology* **77**, 263-267.

Souilem, O (1986) Contribution à l'étude des gongylonèmes chez les bovins du Cap-Bon. *DVM Thesis. Sidi Thabet. Tunisia.*

Syrjälä, P, Oksanen, A, Hälli, O, Peltoniemi, O and Heinonen, M (2010) *Metastrongylus* spp. infection in a farmed wild boar (*Sus scrofa*) in Finland. *Acta Veterinaria Scandinavica* **52** (Suppl 1), S21. http://doi.org/10.1186/1751-0147-52-S1-S21

Thienpont, D, Rochette, F and Vanparijs, OFJ (1986) Diagnosing helminthiasis by coprological examination. *Janssen Research Fundation*, Beerse, Belgium. 187 pp.

Torgerson, PR and Heath, DD (2003) Transmission dynamics and control options for *Echinococcus granulosus*. *Parasitology* **127** Suppl, S143-158.

Umhang, G, Richomme, C, Hormaz, V, Boucher, JM and Boué, F (2014) Pigs and wild boar in Corsica harbor *Echinococcus canadensis* G6/7 at levels of concern for public health and local economy. *Acta Tropica* **133**, 64-68. https://doi.org/10.1016/j.actatropica.2014.02.005

Table 1. Prevalence, intensity and abundance of pulmonary, hepatic and gastrointestinal helminth species in Tunisian wild boar.

Parasites	Examined	Infected	P (%) (± SD)	Total parasites	Intensity	Abundance
Pulmonary and hepatic helminths						
Echinococcus granulosus larvae	297	56	18.9 ± 0.023	257	4.58	0.86
Metastrongylus sp.	297	179	60.3 ± 0.028	19 220	107.37	64.71
Taenia hydatigena larvae	297	13	4.4 ± 0.011	13	1	0.04
All	297	200	67.3 ± 0.027	19490	97.45	65.62
Gastrointestinal helminths						
Gongylonema pulchrum	264	5	1.9 ± 0.008	7	1.4	0.02
Physocephalus sexalatus	264	9	3.4 ± 0.011	74	8.22	0.28
Ascarops strongylina	264	145	54.9 ± 0.031	6804	46.92	25.77
Gnathostoma hispidium	264	1	0.4 ± 0.004	1	1	0.003
Globocephalus urosubulatus	264	70	26.5 ± 0.027	2410	34.42	9.12
Macracanthorhynchus hirudinaceus	264	163	61.7 ± 0.030	319	1.47	1.2
Brachylaemus suis	264	56	21.2 ± 0.025	2953	52.73	11.18
Hymenolepis diminuta	264	1	0.4 ± 0.004	2	2	0.007
All	264	212	80.3 ± 0.024	12570	59.29	47.61
Global infection	561	412	73.5 ± 0.019	32060	77.81	57.14

SD : standard deviation

Table 2. Prevalence, intensity, range and abundance of visceral helminth species per organ in Tunisian wild boar.

Location and helminths	Prevalence (%)	Intensity	Abundance	Range	Parasite number	Examined W.B.	Infected W.B.	Frequency of infection/organ
Lungs								60.3%
E granulosus larvae	12.5	3.54	0.44	1 to 8	131	297	37	
Metastrongylus apri	60.3	123.51	34.10	1 - 234	10 128	297	179	
Metastrongylus pudendotectus	60.3	93.73	30.61	1 - 200	9 092	297	179	
Liver								0.4%
Echinococcus granulosus larvae	12.1	3.5	0.42	1 to 29	126	297	36	
Taenia hydatigena larvae	4.4	1	0.04	0 to 1	13	297	13	
Esophagus								0.02%
Gongylonema pulchrum	1.9	1.4	0.02	1 to 2	7	264	5	
Stomach								21.5%
Physocephalus sexalatus	3.4	8.22	0.28	2 to 18	74	264	9	
Ascarops strongylina	54.9	46.92	25.77	1 to 495	6 804	264	145	
Gnathostoma hispidium	0.4	1	0.003	0 to 1	1	264	1	
Small intestine								17.7%
Globocephalus urosubulatus	26.5	34.42	9.12	1 to 91	2410	264	70	
Macracanthorhynchus hirudinaceus	61.7	1.95	1.2	1 to 18	319	264	163	
Brachylaemus suis	21.2	52.73	11.18	1 to 564	2953	264	56	
Hymenolepis diminuta	0.4	2	0.007	0 to 2	2	264	1	

Table 3. Prevalence of visceral helminths in wild boar from northeast and northwest Tunisia.

Parasites	NorthEast			North West				NorthEast		NorthWest	
	Examined	Infected	P (%)	Examined	Infected	P (%)	χ2 / F.E.T	Nb parasites	Intensity	Nb parasites	Intensity
Pulmonary and hepatic helminths											
E granulosus larvae	119	23	19.3	178	33	18.5	χ2= 0.028; p= 0.86	79	3.43	178	5.39
Metastrongylus sp.	119	82	68.9	178	97	54.5	χ2= 6.18; p= 0.01*	7695	93.84	11525	118.81
Taenia hydatigena larvae	119	8	6.7	178	5	2.8	χ2= 2.6; p= 0.1	8	1	5	1
All	119	87	73.1	178	113	63,5	χ2= 3; p= 0.08	7782	89.44	11708	103.61
Gastrointestinal helminths											
Gongylonema pulchrum	186	1	0.5	78	4	5.1	F.E.T; p= 0.02*	1	1	6	1.5
Physocephalus sexalatus	186	6	3.2	78	3	3.8	F.E.T; p= 0.72	58	9.66	16	5.33
Ascarops strongylina	186	89	47.8	78	56	71.8	χ2= 12.72; p= 0.0003*	1880	21.12	4924	87.92
Gnathostoma hispidium	186	1	0.5	78	0	0	F.E.T; p= 1	1	1	0	0
Globocephalus urosubulatus	186	54	29.0	78	16	20.5	χ2= 2.04; p= 0.15	1903	35.24	507	31.68
Macracanthorhynchus hirudinaceus	186	42	22.6	78	43	55.1	χ2= 26.66; p= 2.41	183	4.35	136	3.16
Brachylaemus suis	186	36	19.3	78	20	25.6	χ2= 1.29; p= 0.25	1290	35.83	1663	83.15
Hymenolepis diminuta	186	1	0.5	78	0	0	F.E.T; p= 1	2	2	0	0
All	186	136	73.1	78	76	97.4	χ2= 20.54; p= 0.0000*	5318	39.10	7252	95.42
Global infection	305	223	73.1	256	189	73.8	χ2= 0.036; p= 0.84	13100	42.95	18960	100.31

* Significant ; P : prevalence ; Nb : number

Table 4. Prevalence and intensity of helminth species in Tunisian wild boar relative to host age.

Parasites	Age (years)						
	< 2		2 to 3		≥ 4		
	P (%) (± S.D.)	Intensity	P (%) (± S.D.)	Intensity	P (%) (± S.D.)	Intensity	Prevalence p value
Pulmonary and hepatic helminths							
E. granulosus larvae	10.4 (±0.029)	4,25	21 (±0.042)	6,05	27.6 (±0.048)	3,54	$\chi2 = 9.96$; p= 0.006*
Metastrongylus sp.	46.1 (±0.046)	64.28	67.3 (±0.048)	75.54	71.3 (±0.049)	177.06	$\chi2 = 16.05$; p= 0.0003*
Taenia hydatigena larvae	4.3 (±0.019)	1	6.3 (±0.025)	1	2.3 (±0.016)	1	F.E.T; p= 0.46
All	58.3 (± 0.046)	51.68	63.2 (±0.049)	82.7	83.9 (±0.039)	151.56	$\chi2 = 15.92$; p= 0.0003*
Gastrointestinal helminths							
Gongylonema pulchrum	0 (±0.00)	0	3.5 (±0.017)	1.25	1.8 (±0.018)	2	F.E.T; p= 0.18
Physocephalus sexalatus	5.2 (±0.022)	10.6	2.6 (±0.015)	6.33	1.8 (±0.018)	2	F.E.T; p= 0.55
Ascarops strongylina	50.5 (±0.051)	34.57	59.3 (±0.046)	45.41	53.7 (±0.068)	71.27	$\chi2 = 1.66$; p= 0.43
Gnathostoma hispidium	0 (±0.00)	0	0.9 (±0.009)	1	0 (±0.00)	0	F.E.T; p= 1
Globocephalus urosubulatus	25.8 (±0.044)	20.4	19.5 (±0.037)	55.63	42.6 (±0.067)	29.39	$\chi2 = 10.26$; p= 0.005*
Macra hirudinaceus	25.8 (±0.044)	4.4	38.9 (±0.046)	3.72	29.6 (±0.062)	2.81	$\chi2 = 4.34$; p= 0.11
Brachylaemus suis	28.9 (±0.046)	27.25	15 (±0.034)	110.35	20.4 (±0.055)	28.54	X2= 5.99 ; p= 0.04*
Hymenolepis diminuta	1 (± 0.01)	2	0	0	0	0	F.E.T; p= 0.57
All	76.3 (±0.043)	42.32	80.5 (±0.037)	69.58	87 (±0.046)	66.08	$\chi2 = 2.54$; p= 0.28
Global infection	66.5 (± 0.032)		72.6 (± 0.031)		85.1 (± 0.030)		$\chi2 = 15.13$; p= 0.0005*

* Significant; P: prevalence; S.D.: Standard Deviation

Table 5. Frequency distributions of wild boar parasites and association with age or geographical region.

Parasite	Frequency distribution	Abundance (CI)	Association of abundance with age or region	Zero Inflation	Association of zero inflation parameter with age or region	Prevalence (CI)	Association of prevalence with age or region
Ascarops sp	Negative Binomial	25.8 (19.3-35.5)	NS (age) Higher abundance in north west (AR 6.23, $p < 0.0001$	NA	NA	0.59 (0.49-0.61)	NS (age), increased odds of infection north west (OR= 2.77)
Brachylaemus sp	Negative binomial	11.2 (6.6-21.4)	NS	NA	NA	0.21 (0.166-0.264)	NS
Echinococcus granulosus	Negative binomial	0.89 (0.59-1.34)	Increases with age, nonlinear (see text)	NA	NA	0.19 (0.15-0.24)	Increases with age, nonlinear (see text)
Globocephalus sp	Zero inflated negative binomial	9.1 (7.8-10.5)	NS	0.66 (0.55-0.76)	Decreases with age (OR 0.75, p=0.017)	0.27 (0.21-0.32)	Increases with age (OR 1.29, p=0.013)
Gnathostoma spp	Poisson^	0.0038 (0.0002-0.017)	*	*		0.0038 (0.0002-0.017)	*
Gongylonema spp	Negative binomial	0.027(0.009-0.073)	*	*		0.019 (0.0068-0.040)	*
Hymenolepis sp	Negative binomial	0.008(0.0007-0.26)	NA	NA	NA	0.0038 (0.0002-0.017)	*
Macracanthorhynchus sp	Zero inflated negative binomial	1.21 (0.92-1.63)		0.55 (0.41-0.69)	NS	0.32 (0.27-0.38)	NS
Metastrongylus sp	Zero inflated negative binomial	64 (56-74)	Increases with age, AR = 1.17, $p<0.0001$	0.53 (0.34-0.45)	Decreases with age (OR 0.75, $p <0.0001$), higher in North West (OR 1.84, p=0.018)	0.71 (0.65-0.76)	Lower in north west (OR 0.51, p=0.014)

Physocephalus sp	Negative binomial	0.28 (0.10-1.23)	*	0.034 (0.017-0.061)	*
Taenia hydatigena	Poisson^	0.044 (0.024-0.072)	*	0.044 (0.024-0.071)	*

AR = Abundance ratio

OR = odds ratio

^ Positive animals only had single parasites, so no meaningful analysis of other frequency distributions was possible.

* insufficient positive animals to evaluate

Table 6. Prevalence, intensity and abundance of pulmonary and hepatic helminths in Tunisian wild boar relative to host gender.

Parasite	Female						Male						P value	Student test
	Exam	Inf	P (%)	Nb parasites	I	A	Exam	Inf	P (%)	Nb parasites	I	A	(Prevalence)	(Abundance)
E. granulosus larvae	134	29	21.6 ± 0.036	109	3.75	0.81	163	27	16.6 ± 0.029	148	5.48	0.9	$\chi2$= 1.23; p= 0.26	P= 0.83
Metastrongylus sp.	134	83	61.9 ± 0.042	9 836	118.5	73.4	163	96	58.9 ± 0.039	9 337	97.26	57.28	$\chi2$= 0.28; p= 0.59	p= 0.12
T. hydatigena larvae	134	6	4.5 ± 0.018	6	1	0.044	163	7	4.3 ± 0.016	7	1	0.042	$\chi2$= 0.005; p= 0.93	P= 0.93
All	134	108	80.6 ±0.034	9951	92.13	74.26	163	119	73 ± 0.035	9492	79.76	58,23	$\chi2$= 2.35; p= 0.125	p = 0.128

Exam: examined; Inf: infected; I: Intensity; A: Abundance

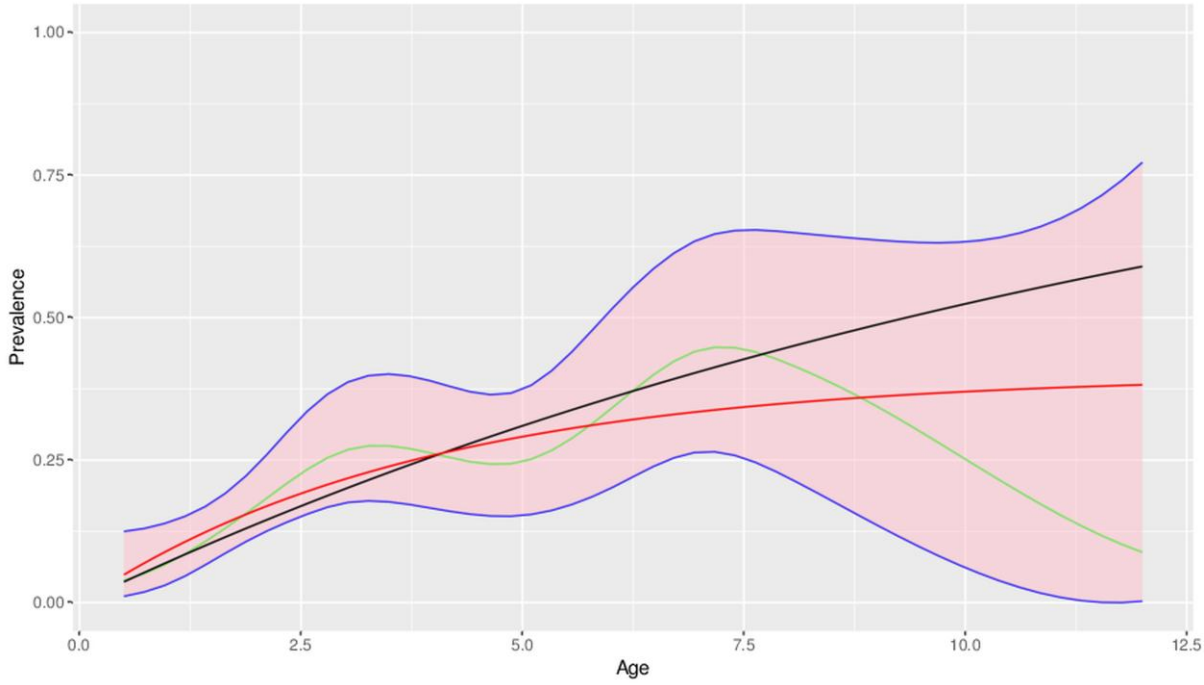

Figure 1. Variation of prevalence with age for *Echinococcus granulosus*. Best fit generalized additive model (GAM) (green) with 95% confidence limits (pink). Predicted models in the absence (black line) and presence (red line) of parasite-induced immunity.

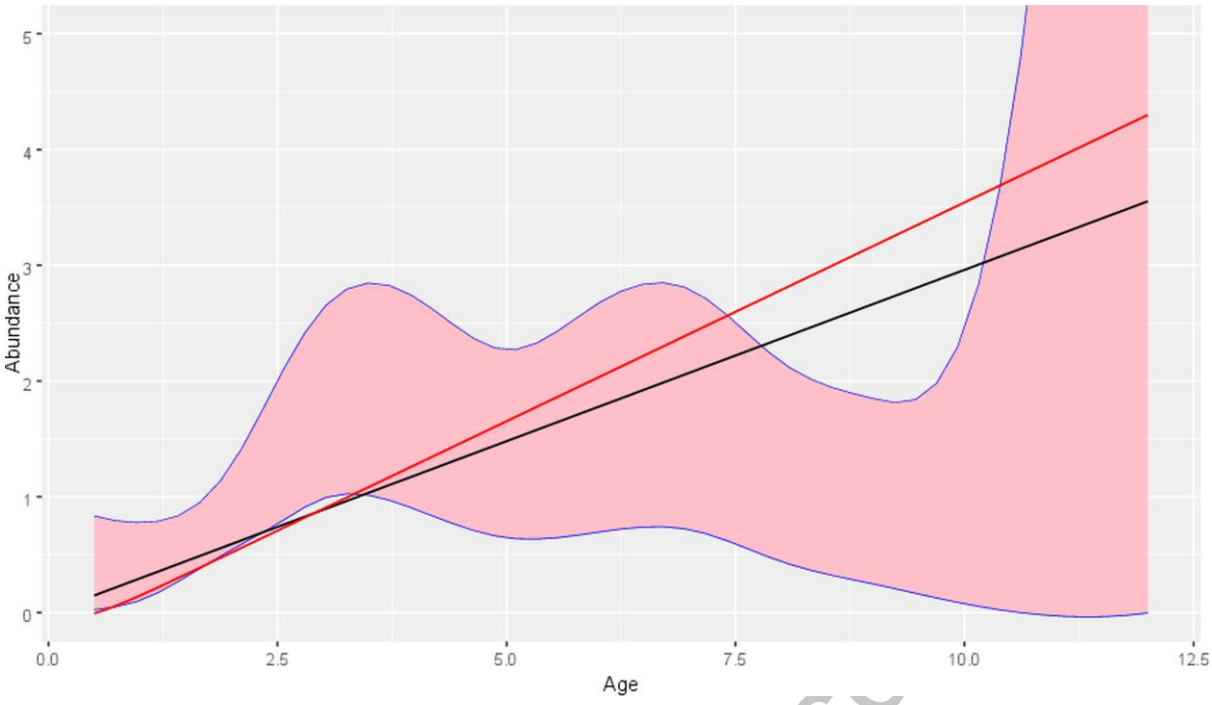

Figure 2. Variation of abundance with age for *Echinococcus granulosus*. Best fit generalized additive model (GAM) (green) with 95% confidence limits (pink). Predicted models in the absence (black line) and presence (red line) of parasite-induced immunity.